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PROTOZOA IN A CASE OF TROPICAL ULCER ("DELHI SORE").

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The clinical and anatomical features of this peculiar skin disease are fully described in any modern text-book of dermatology, so that it is not necessary to give any lengthy description of them here. In brief, the disease consists of single or multiple focal lesions of the skin characterized by the formation of elevated, indurated areas, which ulcerate and eventually cicatrize. They have considerable resemblance to certain forms of cutaneous tuberculosis and syphilis. The lesions occur usually on parts not protected by clothing. Histologically the lesions consist in infiltration of the corium and subcutaneous tissue by cells, together with hypertrophy, atrophy, and disappearance of the epidermis. The affection lasts for months, or for a year, or longer. It is endemic only in tropical and sub-tropical countries. It is generally believed to be of an infectious nature and is capable of transmission from one individual to another by inoculation, but apparently is not contagious in the usual meaning of that term. There is evidence that mosquitoes and other insects may be the carriers of the infectious agent. The infectious agent has been sought for by a considerable number of observers, but with only negative, or contrary, or inconclusive results. Fungi, bacteria, and protozoa have been described in the lesions. The French observers particularly have written much concerning the occurrence of micrococci in connection with the disease, but have not offered convincing proof of their significance. At the present time no micro-organism has been satisfactorily shown to be the causal agent in the disease.

From an examination of the literature on the subject it seems that the results of only three observers are worthy of serious consideration in this paper.

D. D. Cunningham[1] in 1885 published the results of his examination of a specimen of "Delhi boil" that had been placed in alcohol immediately after its removal from the patient in Delhi. The epidermis over the specimen was intact and there was no evidence of ulceration. He studied frozen sections of the specimen, stained in various ways, and found that the condition was one of extensive cellular infiltration of the corium and subcutaneous tissue together with atrophy of the epidermis in some places and hyperplasia in others. In sections which had been stained with gentian violet and then considerably decolorized with alcohol he found a large number of peculiar bodies which he describes as follows:

"They varied very considerably in size. The average diameters of a series of measured specimens were 12.6 μ by 8.8 μ, the largest measuring 12.8 μ by 25.6 μ, the smallest 6.4 μ by 6.4 μ. Such minute specimens as the latter were, however, rare, and as a rule they were considerably larger than the lymphoid elements among which they were situated. Their form also varied greatly. In some cases they were circular, in others elliptical, in others irregularly lobate. Their contour was in the majority of instances smooth, but in some of a more or less tuberculate character. In some specimens a very delicate cell wall was clearly visible. In others it was wholly unrecognizable or only to be detected on careful and special scrutiny. The distinctness with which they appear in sections treated with gentian violet is due to the elective staining of the nucleoid bodies which they contain by the dye. The number of such bodies present in different cells varies extremely, — in some cases only a single great nucleoid mass is present, seemingly occupying almost the entire cell body, in others a few of very various sizes occur, and in still others a large number of minute and fairly equal sized ones were thickly scattered throughout the entire cell. The cytoplasm in the gentian violet specimens remains almost uncolored; in those in which

[1] On the Presence of Peculiar Parasitic Organisms in the Tissue of a Specimen of Delhi Boil. Scientific Memoirs by Medical Officers of the Army of India, Part 1, 1884, Calcutta, 1885.

fuchsin has likewise been employed it frequently shows a more or less pronounced red hue. The tuberculate appearance presented by some of the cells is due to the numbers and sizes of the nucleoid bodies present in them, which in association form a mulberry-like mass pressing upon the cell wall and molding it to the inequalities of its surface. Such tuberculate bodies on superficial examination present certain points of resemblance to the characteristic bodies in cases of actinomycosis. On closer examination, however, it is evident that they correspond structurally with the description given above and are not due to any radiate aggregation of filaments. In certain cases appearances apparently corresponding with the occurrence of processes of cell division are present, the bodies of the cells being strongly constricted so as to form two lobes connected by a narrow neck, or two distinct cells occurring, which, from their relations to one another and the character of their opposed surfaces, seem to have just arisen, due to completion of such a process. In many instances, too, a distribution of the cells in little groups separated from one another by comparatively wide areas of granulation tissue can be recognized and may possibly be indicative of the antecedent occurrence of processes of division. The individual cells in some cases are closely packed among the surrounding lymphoid elements; in a large number of instances, however, they appear to lie in a limited clear space. This appearance may possibly be an artificial one, arising as the result of shrinking during the course of preparation. The number of cells visible in individual sections and in different parts of the same section varies considerably. In some sections as many as 80 or even more may be visible at once in a single field under a power of 140 diameters. In others they are present in varying but smaller numbers, and in almost any entire fields may in certain places fail to show any at all.

"After very careful consideration of the features presented by all the various forms present in the tissues, I am inclined to regard them as representing various stages of some simple organism of Mycetozoic nature. In the most

recent systematic treatise dealing with the Mycetozoa or Myxomycetes — Zopf's 'Schleimpilze' — they are subdivided into Monadinæ and Eumycetozoa, and it is to the former group that the organism here dealt with appears to me probably to belong. The appearances presented by the various forms are, according to this view, to be regarded as corresponding to various stages of the development, and, especially, of the development of the Zoöcysts or Sporocysts, of some Monadinic organism. Comparing the characters of the various specimens with one another, we have apparently to deal with the development of parent plasmodia or amebæ, which multiply by division and in which sporoid bodies are gradually developed, the process terminating in some cases with the formation of one great spore, in others with that of a dense aggregate of smaller ones."

It will be obvious to a histologist, from the reading of Cunningham's description of the bodies, that the morphological evidence adduced in favor of their parasitic nature is not sufficient to overcome the objection that they are elements of the tissue or degeneration products. This objection has the greater weight in view of the fact that Cunningham's histological technique was crude and not adapted to permit an adequate examination of the tissue, for he states that he made his observations on frozen sections. The plates that accompany the paper do not show any more morphological detail than is described in the text. It seems possible, however, that Cunningham did see among the various bodies that he describes the large cells described below, and that these large cells were what he regarded as "parent plasmodia" containing small spores.

Gustav Riehl,[1] in a paper published in 1886, reported the result of his examination of a single case. He describes among the infiltrating cells of the lesion many large epithelioid cells containing in their cytoplasm many bodies which he regarded as micrococci, with capsules, frequently more than twenty in a single cell. He regarded the bodies described by Cunningham as degeneration products.

[1] Zur Anatomie und Ætiologie der Orientbeule. Vierteljahrsschrift für Dermatologie und Syphilis, 1886, p. 805.

R. H. Firth,[1] in 1891, states in a short paper that he could confirm the findings of Cunningham. This paper is but little more than a declaration that the author had seen in the lesions of the disease the same bodies described by Cunningham, and it contains nothing more convincing of their parasitic nature than does the paper of Cunningham. He proposed for them the name "Sporozoa furunculosa."

The case of tropical ulcer which is reported in this paper entered the service of Dr. R. B. Greenough in the Out-Patient Department of the Massachusetts General Hospital, July 28, 1903. The patient was a female child, nine years of age, born in Armenia. She presented in the skin of the left cheek, near the mouth, a firm, circular, elevated area, about twelve millimeters in diameter, covered with a blackish scab. This lesion had made its first appearance before the child left Armenia, some two or three months before. Through consultation with Dr. Charles J. White of the Dermatological Department of the hospital, the diagnosis of aleppo boil or tropical ulcer was made. The lesion was excised and curetted by Dr. Greenough, and the material thus obtained, consisting of a piece of grayish translucent tissue about ten millimeters in diameter, and two or three smaller pieces of similar tissue, was immediately given to the writer for examination. Smear preparations were made from this material by rubbing and squeezing pieces of it against the coverglass, so as to cause a deposit of the tissue juices thereon. The smears were then immediately fixed and afterwards stained in various ways. In these preparations peculiar bodies were more or less distinctly visible. They were most clearly seen and differentiated from cell detritus in preparations fixed in pure methyl alcohol, and afterwards stained with the Romanowsky staining fluid for blood films described by the writer in a paper published in a previous volume of this Journal. ("A Rapid Method for the Differential Staining of Blood Films and Malarial Parasites." Journal of Medical Research, Vol. VII., No. 1, January, 1902.) After fixation by the methyl

[1] Notes on the Appearance of Certain Sporozoon Bodies in the Protoplasm of an "Oriental Sore." British Medical Journal, Jan. 10, 1891, p. 60.

alcohol the staining fluid was immediately applied to the preparation without washing in water, or allowing it to dry, and the process of staining continued essentially as described for blood films and malarial parasites in the paper referred to above. The best results were obtained in a preparation fixed with methyl alcohol for only a few minutes.

In the thinner and better fixed and stained portions of smears prepared in this way the peculiar bodies present the following characteristics: They are generally round, sharply defined in outline, and two to four micromillimeters in diameter. A large part of their peripheral portions is stained a pale robin's egg blue, while their central portions are unstained or white. A very prominent feature is the presence in each of the bodies of a larger and a smaller lilac-colored mass. The larger mass is about one-fourth or one-third the size of the body, is of variable shape, but always forms a part of the rounded periphery of the body. The smaller mass in some instances is round, in others is rod-shaped, and in the latter case is of a deeper lilac color than the larger mass. It is usually situated near or at the blue-stained periphery of the body. The blue peripheral portions of the bodies are usually sharply defined from the central unstained portion and sometimes show small unstained areas. A few of the bodies are oval or elongate in form. This is thought to be due to distortion in making the preparation, because in thin sections of the tissue such forms are not apparent. In the thicker portions of the smears the central portion of the bodies is stained blue as well as the periphery.

These bodies are present in very large numbers in the smears, often occurring in aggregations associated with a large nucleus, thus suggesting that they have been contained in a large cell whose outlines have disappeared in the process of fixing and staining. That this is true is shown in the sections of the tissue described below. (See Pl. XXVII., Fig. 2, and Pl. XXVIII., Fig. 3.)

The general morphology of the bodies and the large numbers of them visible in a single field of the microscope, under a high magnifying power, are indicated in Pl.

XXVIII., Fig. 5; Pl. XXIX., Figs. 6, 7, 8, and 9; and Pl. XXX., Figs. 10, 13, 14, and 15.

The constant morphology and structure of these bodies, the differential staining of their parts, their great numbers, and their position in cells seem to justify the belief that they are microörganisms and that they are the infectious cause of the lesion. Assuming that they are microörganisms, it seems reasonable to regard them as protozoa, because of their morphology and staining peculiarities. As to their classification among the protozoa, I am unable to give a definite opinion. Their small size, their great number, their intracellular position, and their morphology suggest that they are microsporidia. Nothing, however, was observed that suggested the developmental or reproductive cycle so characteristic of that group. On the contrary, certain appearances are observed in a few microörganisms, usually of larger size, which suggest multiplication by fission, which is a mode of multiplication apparently unknown among the microsporidia. These appearances consist chiefly in increased size and length of the lilac-colored masses with constriction in their middle parts, and in the presence in a single microorganism of two of the larger or two of the smaller masses or two of each (Pl. XXX., Figs. 10, 11, 12, 13, 14, and 16). In two or three of these microörganisms a process is seen extending inward from the peripheral portion and tending to mark the body into two symmetrical halves (see Pl. XXX., Figs. 10 and 11). Assuming that the lilac-colored masses are of the nature of nuclei, this duplication of them may be regarded as the preliminary process of division of the microorganism into two individuals.

I propose as the generic and specific names for this parasite *Helcosoma tropicum*. The generic name is derived from ἕλκος, a sore.

I do not adopt the name *Sporozoa furunculosa* that Firth applied to the supposed protozoan described by Cunningham in this disease, because that was an ameba-like, spore-forming organism and was obviously different from the one here described.

Microscopical examination of paraffin sections of some of the material which had been fixed in Zenker's fluid gave the following results: The lesion consists essentially of a very extensive infiltration of the corium and papillæ by cells, accompanied by atrophy and disappearance of the epidermis of the part (see Pl. XXVII., Fig. 1). The infiltrating cells are plasma cells, various kinds of lymphoid cells, and large cells with single vesicular nuclei and a relatively large amount of cytoplasm in which are large numbers of the microörganisms. These large cells, over extensive areas, are very numerous and constitute the principal part of the infiltration (see Pl. XXVII., Fig. 2, and Pl. XXVIII., Fig. 3). They are regarded as proliferated endothelial cells. The microörganisms are generally closely packed together throughout the cytoplasm of these cells and occupy most of the available space between the nucleus and the cell membrane. They are almost exclusively in these cells. Many cells contain twenty or more microörganisms. Only in very thin sections, cut with the aid of the Minot-Blake microtome, can the morphology of the individual microörganisms be clearly made out. In these thin sections all the microörganisms appear to be of spherical form, the cortical or peripheral portions staining faintly with nuclear stains and the principal portion of the body remaining unstained, while the larger and smaller lilac-stained masses described in the smear preparations stain deeply with methylene blue and gentian violet. (Pl. XXVIII., Fig. 4, shows three of the microörganisms in focus in a thin section.)

In thicker sections the microörganisms may give to the large cells in which they are situated the appearance of containing numerous basic staining granules of about the size of ordinary pus cocci, each surrounded by a clear space. These granules are the larger nucleus-like masses of the microörganism. The appearances are such as to make it certain that the cells containing micrococci with capsules, described by Riehl in his case and referred to above, were these same cells (see Pl. XXVIII., Fig. 3). As has been pointed out before, it seems possible that these same cells were seen

by Cunningham and by Firth, and represent some of the supposed plasmodia in process of sporulation described by them.

A part of the material was also used for the inoculation of a rabbit by subcutaneous injection, and by the scarification of the skin and of the cornea. No pathogenic effect was noted in the animal.

A small amount of the material was placed in a small quantity of freshly drawn human blood and kept in the incubator for some days. No evidence of multiplication of the microörganisms was obtained.

I wish to acknowledge my obligations to Dr. Robert B. Greenough for affording me the opportunity of studying this case, and to Dr. Charles J. White for helping me in the examination of the literature of the disease.

The interest aroused in the subject of protozoa in disease, by the work of Dr. W. T. Councilman and his pupils, has greatly stimulated the study of this case.

(For the benefit of those who may wish to apply the method used to other cases of tropical ulcer and who have not access to the paper referred to on p. 476, the following directions for the preparation of the staining fluid and for its application to smear preparations from the lesions are given:

Preparation of the Staining Fluid. — Dissolve 0.5 grm. of sodium bicarbonate in 100 ccm. of distilled water, and add to it 1 grm. of methylene blue (Grübler). Steam the mixture in an ordinary steam sterilizer for one hour, counting the time after "steam is up." The heating should not be done in a pressure sterilizer, nor in a water bath, nor in any other way than as stated. When cool, pour the mixture into a large vessel and add to it, stirring or shaking meanwhile, 500 ccm. of a one to one thousand aqueous solution of eosin (Grübler, yellowish, water soluble). In the mixture thus formed a fine blackish precipitate will be visible in suspension, and on the surface a scum with yellowish metallic luster will have appeared. Filter the mixture, collect the precipitate on the filter paper and allow it to dry thereon without washing. When thoroughly dry, dissolve this precipitate in pure methyl alcohol in a proportion of 0.5 grm. to 100 ccm. of alcohol. This alcoholic solution is the staining fluid. It will keep indefinitely, as will also the dry precipitate. Precautions should be taken to prevent the alcohol from evaporating, for thus the solution may become too saturated and precipitates may form on the preparation in the process of staining. If the staining fluid deposits such precipitates, it should be filtered and a small quantity of methyl alcohol added to it.

Method of Applying the Staining Fluid. — Place the fresh cover-glass preparation in pure methyl alcohol and allow it to remain therein for two or three minutes. It is probably best that the preparation be allowed to dry in the air before placing it in alcohol. Next remove the preparation from the alcohol, grasp it with coverglass forceps, and, without permitting

it to dry, pour onto it as much of the staining fluid as the cover-glass will conveniently hold, and allow the fluid to remain one minute. Then add water to the staining fluid drop by drop until a delicate scum with iridescent metallic luster becomes visible on its surface. Avoid diluting the fluid more than enough to just cause this scum to appear. If the staining fluid has been properly prepared, this scum will form before the fluid has been diluted enough to be transparent. The diluted fluid is to remain on the preparation for three minutes. During this time the most important part of the staining is effected. After this the preparation is to be washed with water until the nuclei of cells in the better-spread portions of the preparation appear well differentiated under a low power of the microscope and until any red blood corpuscles present have a yellowish or pinkish color. This will probably require about a minute's washing. The washing in water is important, for it removes superfluous blue stain and brings out the differential staining of the elements in the preparation. Distilled water should be used, for tap water may spoil the staining. The quality of the staining and the progress of the differentiation can be easily judged by placing the preparation, film-side uppermost, on a slide and examining it with a Zeiss AA or similar objective. When the decolorization is judged sufficient, the preparation is to be thoroughly dried and mounted in balsam. Dried stain adherent to the upper side of the coverglass may be easily removed with alcohol. The nuclei of cells should have a blue or deep lilac color and red blood corpuscles a pink or orange color. The cytoplasm of polynuclear leucocytes should show lilac-colored granules and the cytoplasm of lymphocytes should have a robin's egg blue color, while the protozoa should have the color appearances described.)

DESCRIPTION OF THE PLATES.

(Photographs by Mr. L. S. Brown, Clinico-Pathological Laboratory, Massachusetts General Hospital.)

PLATE XXVII.

FIG. 1. A section of the lesion under a low magnifying power, showing the extensive cellular infiltration of the dermis and the atrophy of the epidermis.

FIG. 2. A section of the lesion, showing the general character of the infiltrating cells. The granular appearance of the large cells is due to the presence of the microörganisms within their cytoplasm. x 500 approx.

PLATE XXVIII.

FIG. 3. A section of the lesion, showing the large cells containing the microörganisms under a higher magnification than Fig 2. x 1,000 approx.

FIG. 4. A very thin section of the lesion cut on the Minot-Blake microtome, showing the morphology of three of the microörganisms. The indefinite mass in the center of the figure is made up largely of microorganisms which are out of focus. x 2,000 approx.

FIG. 5. Smear preparation from the lesion stained with Wright's Romanowsky blood-staining fluid. The ring-like bodies with white central portions and containing a larger and a smaller dark mass are the microörganisms. The dark masses in the bodies are stained a lilac color, while the peripheral portions of the bodies, in typical instances, are stained a pale robin's egg blue. The very dark masses are nuclei of cells of the lesion. x 1,500 approx.

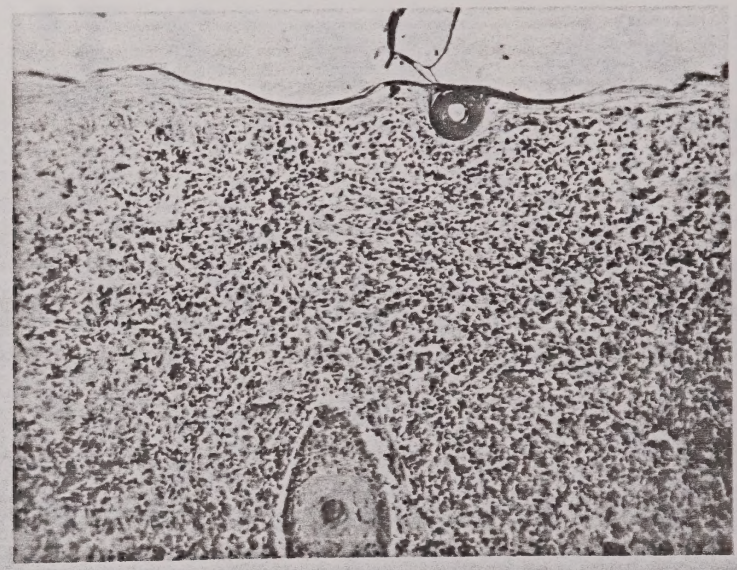
PLATE XXIX.

FIGS. 6, 7, 8, and 9. Smear preparations stained as in the case of Fig. 5 and showing essentially the same things. All x 1,500 approx.

PLATE XXX.

FIGS. 10, 11, 12, 13, 14, 15, and 16. All are smear preparations stained as described for the preceding figures. All x 1,500 approx.

FIGS. 10, 11, 12, 13, 14, and 16 show the elongation, constriction, and duplication of the lilac-stained, dark appearing bodies in the microörganisms described in the text.



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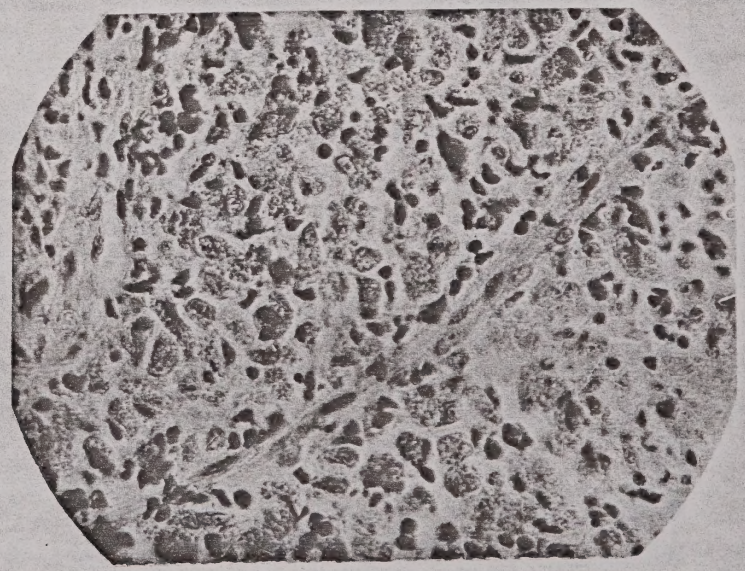

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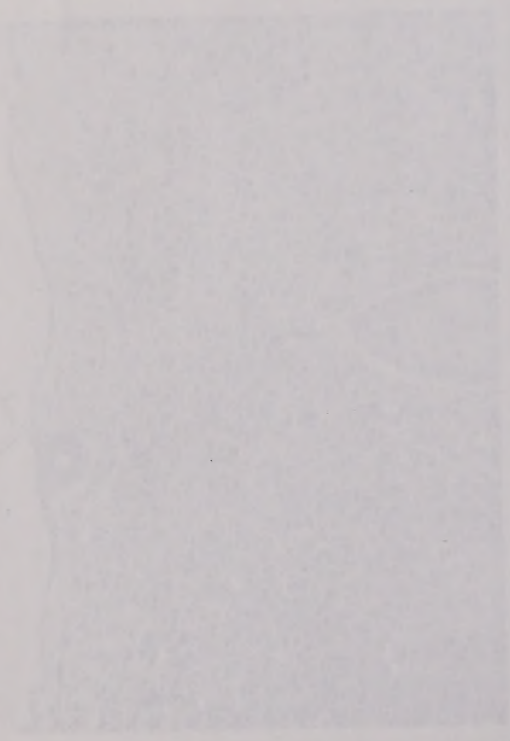

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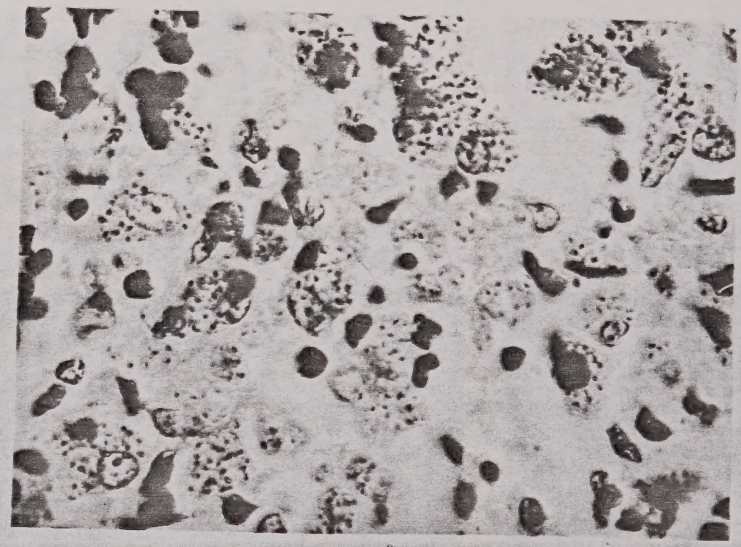

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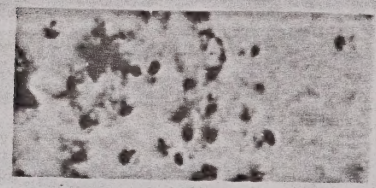

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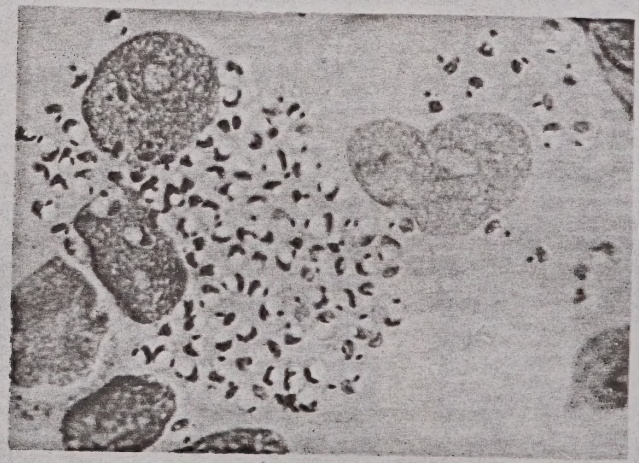

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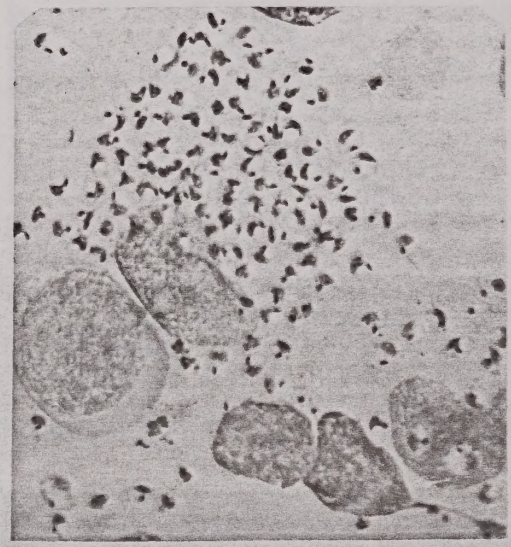

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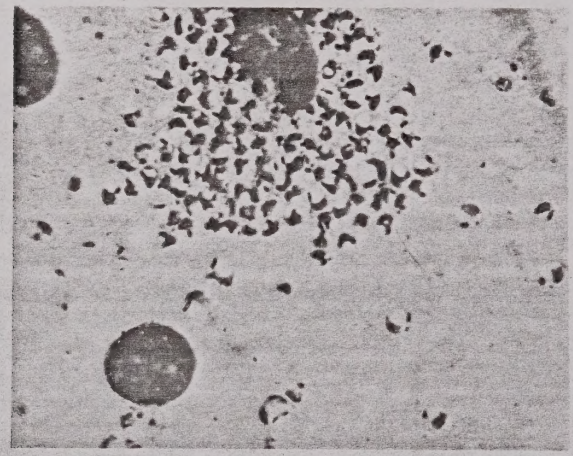

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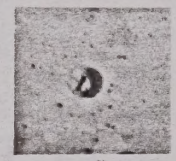

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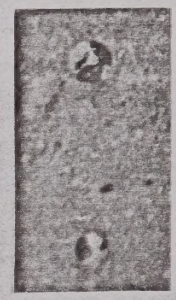

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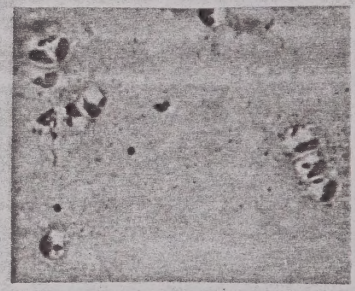

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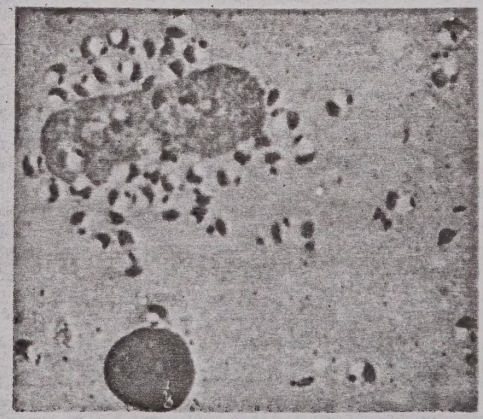

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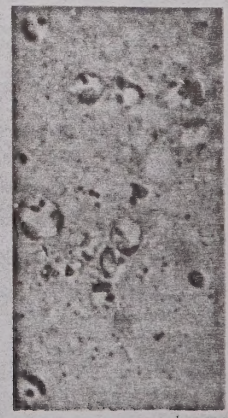

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